Journal of Pharmaceutical, Chemical and Biological Sciences
ISSN: 2348-7658
Impact Factor (SJIF): 2.092
December 2014-February 2015; 2(4)

Original Research Article

Improved Efficacy of Gliclazide with the Aqueous Extract of *Emblica officinalis* on Pharmacodynamic Activity in Normal and Alloxan Induced Diabetic Rats

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Received: 07 February 2015 Revised: 20 February 2015 Accepted: 24 February 2015

ABSTRACT

The present study was carried out to evaluate the hypoglycemic activity and antihyper glycemic activity of aqueous extracts of whole plant of *Emblica officinalis* in normal and in alloxan induced diabetic rats. The aqueous extract of *Emblica officinalis* (30 mg/kg, 100 mg/kg, and 300 mg/kg bd.wt) and the dose (300 mg/kg) that produced an optimal reduction (30-40%) of blood glucose was selected for the study. Blood samples were collected at desired intervals of time and analyzed for blood glucose by GOD-POD method. Gliclazide a second generation sulfonyl urea was selected for the interaction study with that of the *Emblica officinalis*. The doses of gliclazide (1mg/kg, 2 mg/kg and 4 mg/kg bd.wt) were given to normal and diabetic rats and the dose (2 mg/kg bd.wt) that produced an optimal reduction of blood glucose was selected for the study. The prior administration of *Emblica officinalis* significantly increased the hypoglycemic and antihyperglycemic activity of gliclazide.

Keyword: *Emblica officinalis*; hypoglycaemic; antihyperglycemic activity; Gliclazide

INTRODUCTION

Diabetes (DM) is a chronic metabolic disorder characterized by hyperglycemia caused by defective insulin secretion, resistance to insulin action or combination of both. It is a disease prevailing throughout the world irrespective of age, sex and race. Among diabetics,

approximately 95% of patients have type 2 diabetes mellitus (NIDDM), whereas about 5% of patients have type 1 diabetes mellitus (IDDM). Patients with DM are at risk for microvascular complications like retinopathy, nephropathy and neuropathy and macrovascular complications like myocardial infarction that increase morbidity and mortality [1, 2, 3].

Emblica officinalis Gaertn. (Euphorbiaceae) commonly known as Indian gooseberry or amla, is arguably the most important medicinal plant in the Indian traditional system of medicine, the Ayurveda. All parts of the plant are used in various Ayurvedic/Unani medicine (*Jawarish amla*) herbal preparations, including the fruit, seed, leaves, root, bark and flowers.Various phytochemical constituents identified for hypoglycemic / antihyperglycemic activities are ascorbic acid, beta-sitosterol, epigallocatechin gallate, manganese, niacin, pectin, quercetin, and tryptophan [4, 5, 6].

MATERIALS AND METHODS

Plant extracts

The plant extract of *Emblica officinalis* used for the investigation was obtained from Laila Impex, Vijayawada, Andhra Pradesh, India.

Gliclazide

The Gliclazide used in the study was obtained as gift samples from Dr. Reddy's Laboratories, Hyderabad, Telangana.

Animals

Albino rats (Wistar strain) of either sex procured from Mahaveer Enterprises, Hyderabad, India were used in the study. They were maintained under standard laboratory conditions at ambient temperature of 25±2°C and 50±15% relative humidity with 12 hours light/12 hours dark cycle. Rats were fed with commercial pellet diet (Rayan's Biotechnologies Pvt.Ltd, Hyderabad, India) and water ad libitum. Rats were fasted for 18 hours prior to the experiment, allowing access to water and during the experiment food and water were withdrawn.

Experimental Design

Twelve groups of rats, six in each received the following treatment schedule.

Normal Rats

Group-I : Normal control (water).
Group-II: *Emblica officinalis* (30 mg/Kg body weight, orally)
Group-III: *Emblica officinalis* (100 mg/Kg body weight, orally)
Group-IV: *Emblica officinalis* (300 mg/Kg body weight, orally)
Group-V: Gliclazide (1 mg/Kg body weight, orally)
Group-VI: Gliclazide (2 mg/Kg body weight, orally)
Group-VII: Gliclazide (4 mg/Kg body weight, orally)
Group-VIII: *Emblica officinalis* +Gliclazide (300 mg+2 mg/Kg body weight, orally)

Diabetic Rats

Group-IX: Normal control (water).
Group-X: *Emblica officinalis* (300 mg/Kg body weight, orally)
Group-XI: Gliclazide (2 mg/Kg body weight, orally)
Group-XII: *Emblica officinalis* +Gliclazide (300 mg+2 mg/Kg body weight, orally)

Induction of Diabetes in Experimental Animals

Animals were injected with freshly prepared aqueous solution of alloxan monohydrate in two doses of 100 mg/kg and 50 mg/kg body weight intraperitonial route for two consecutive days [7]. Then 10% dextrose was administered to combat the immediate hypoglycemia. Blood sugar was measured and rats showing fasting blood sugar levels above 250 mg/dL were selected for the study.

Collection of Blood Sample and Blood Glucose Determination

Blood was collected from the retro orbital plexus of rats. The blood samples were collected at 0, 1, 2, 3, 4, 6, 8, 10, and 12 h intervals from all the groups of rats after drug administration and were analysed for blood glucose by GOD/POD method.

Statistical Analysis

All the values of percentage reduction of blood glucose were expressed as mean±standard error of mean (S.E.M.) and analyzed for ANOVA and post hoc Dunnet's t-test. Differences between groups were considered significant at P <0 .01 levels.

RESULTS

Normal Rats

Emblica officinalis induced hypoglycemia was studied by administering it in different doses namely 30,100,300 mg/kg body weight for the dose response effect. *Emblica officinalis* produced 17.81±3.70% and 27.95±2.45% reduction in blood glucose levels with 30 and 100 mg/kg bd.wt at 4 h. *Emblica officinalis* produced 34.14±1.53% reduction in blood glucose levels with 300 mg/kg bd.wt at 3 h.

Gliclazide was used as standard drug as a representative of sulphonylureas in the present study. It was tested at 1, 2, and 4 mg/kg bd.wt doses and 2 mg/kg bd.wt was found to produce optimal reduction in blood glucose. Gliclazide induced hypoglycemia was studied by administering a dose of 2 mg/kg bd wt. Gliclazide produced 34.07±0.54% and 31.28±0.64% reduction in blood glucose levels at 3 h and 8 h respectively.

The influence of 300 mg/kg bd wt of aqueous extract of *Emblica officinalis* was studied on the hypoglycaemic effect of gliclazide (2 mg/kg bd. wt) in normal rats. This combination produced a peak effect of 39.07 ±0.56 % reductions in blood glucose at 2 h and percentage reduction was maintained above 30% from 2 h to 10 h. (Results are represented in table 1-3 and Figure 1-3)

Table 1: Dose response relationship of *Emblica Officinalis* (EO) on % blood glucose reduction in normal rats

Time (h)	Percentage blood glucose reduction in normal rats (Mean±SEM)		
	EO (30 mg/kg)	EO (100 mg/kg)	EO (300 mg/kg)
0	0.00±0.00	0.00±0.00	0.00±0.00
1	12.04±1.00	9.06±1.15	10.64±0.79
2	16.78±2.53	13.63±0.78	22.27±1.22
3	17.34±2.72	18.57±1.39	**34.14±1.53**
4	17.81±3.70	27.95±2.45	27.00±1.23
6	14.53±3.32	16.33±1.52	20.08±1.55
8	10.25±2.53	8.00±1.75	12.61±0.78
10	5.48±1.84	3.57±1.73	7.89±0.80
12	0.81±1.52	0.32±0.61	4.93±0.54

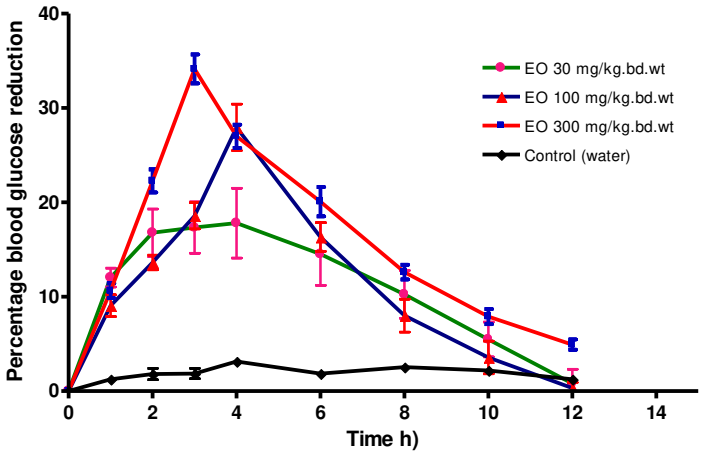

Fig. 1. Dose response relationship of *Emblica officinalis* on % blood glucose reduction in normal rats

Table 2: Dose response relationship of Gliclazide on % blood glucose reduction in normal rats

Time (h)	Percentage blood glucose reduction in normal rats (Mean±SEM)		
	Gliclazide (1 mg/kg)	Gliclazide (2 mg/kg)	Gliclazide (4 mg/kg)
0	0.00±0.00	0.00±0.00	0.00±0.00
1	8.78±1.19	7.36±0.68	18.60±0.58
2	18.82±1.09	19.69±1.14	26.15±0.89
3	29.99±0.80	**34.07±0.54**	35.26±0.31
4	19.27±0.98	23.51±2.39	31.60±2.48
6	14.75±1.17	22.34±1.10	24.94±1.69
8	24.98±1.35	**31.28±0.64**	34.57±0.80
10	12.72±1.06	13.34±0.51	17.70±2.17
12	10.47±0.85	7.36±0.61	7.04±1.73

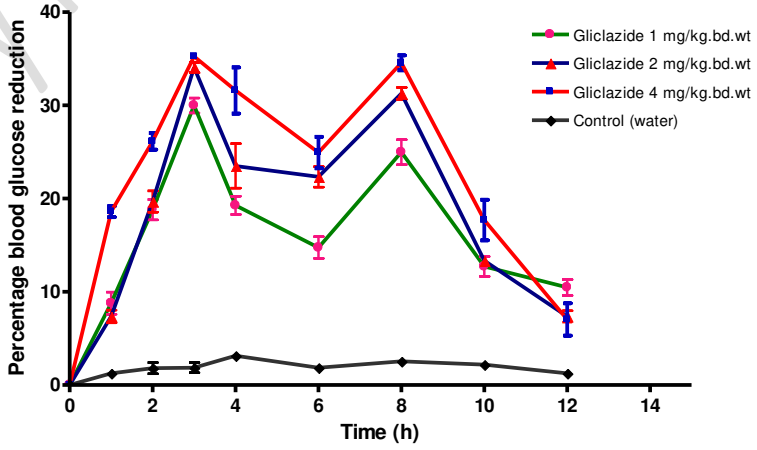

Fig.2. Dose response relationship of Gliclazide on % blood glucose reduction in normal rats

Table 3: The percent blood glucose reduction of Vs time with *Emblica officinalis*, gliclazide and their combination in normal rats

Time (h)	Gliclazide (GL)	*Emblica officinalis* alone	EO + GL
	Mean±SEM	Mean±SEM	Mean±SEM
0	0.00±0.00	0.00±0.00	0.00±0.00
1	7.36±0.68	10.64±0.79	24.62±0.55
2	19.69±1.14	22.27±1.22	**39.07±0.56**
3	**34.07±0.54**	**34.14±1.53**	38.40±0.32
4	23.51±2.39	27.00±1.23	38.07±0.66
6	22.34±1.10	20.08±1.55	34.10±0.73
8	31.28±0.64	12.61±0.78	41.61±2.45
10	13.34±0.51	7.89±0.80	30.67±0.64
12	7.36±0.61	4.93±0.54	23.91±0.60

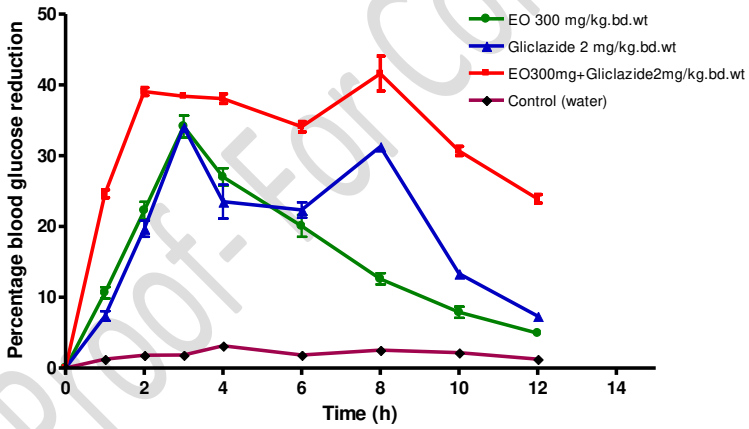

Fig. 3. The percent blood glucose reduction of Vs time with *Emblica officinalis*, gliclazide and their combination in normal rats

Diabetic rats

The aqueous extract of *Emblica officinalis* (300 mg/kg body weight) produced peak effect of 32.47±0.56% reductions in blood glucose at 3 h. Gliclazide 2 mg/kg body weight produced a biphasic response with peak effect of 38.62±0.42% and 35.81±0.19% reduction in blood glucose at 3 h and 8 h respectively.

The selected dose of aqueous extract of *Emblica officinalis* enhanced the anti-hyperglycaemic effect of glyclazide with a peak reduction in blood glucose of 41.20±0.30% at 2 h and 43.13±0.38% at 8 h. The enhanced activity was shown to be sustained from 2 h to 10 h. (Results are represented in table 4 and Figure 4)

Table 4: The percent blood glucose reduction of Vs time with *Emblica officinalis*, gliclazide and their combination in diabetic rats

Time (h)	Gliclazide	Emblica officinalis alone	EO + GL
	Mean±SEM	Mean±SEM	Mean±SEM
0	0.00±0.00	0.00±0.00	0.00±0.00
1	12.46±0.28	4.52±0.75	23.77±0.65
2	21.65±0.51	15.53±0.60	**41.20±0.30**
3	**38.62±0.42**	**32.47±0.56**	40.79±0.27
4	31.87±0.64	27.48±0.85	38.17±0.28
6	27.03±0.50	17.88±0.69	35.55±0.20
8	**35.81±0.19**	12.98±0.51	**43.13±0.38**
10	25.10±0.47	8.08±1.25	31.77±0.47
12	14.05±0.66	5.30±0.58	24.88±0.40

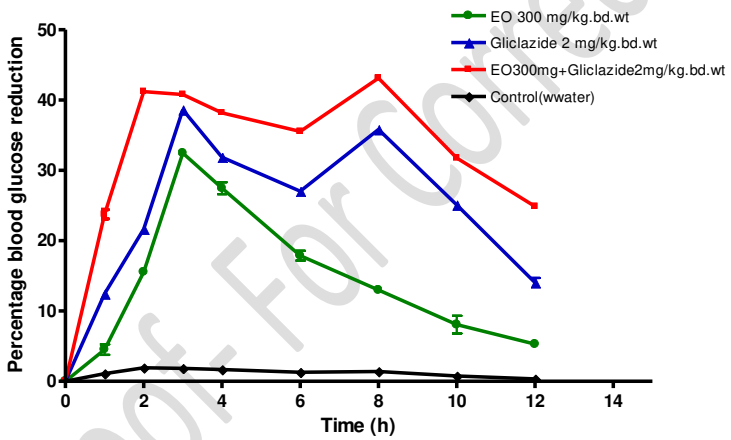

Figure 4: The percent blood glucose reduction of Vs time with *Emblica officinalis*, gliclazide and their combination in diabetic rats

DISCUSSION

The sulphonylureas act by stimulating the release of insulin from pancreatic beta cells of islets of Langerhans and by increasing the sensitivity of peripheral tissues to insulin. The predominant effect of sulfonylureas is on the insulin secretion [8]. Sulphonylureas also stimulate the release of somatostatin, and they may suppress the secretion of glucagon slightly [9]. The reduction in blood glucose with *Emblica officinalis* might be due to the presence of active constituents like ascorbic acid, beta-sitosterol, manganese, niacin, pectin, quercetin, epigallocatechin gallate and tryptophan [4].

The presence of ascorbic acid in EO might also contribute for hypoglycemic action as ascorbic acid was shown to produce hypoglycaemia in normal rats [10]. The prior administration of EO significantly increased the hypoglycemic effect of gliclazide during 1 h to 12 h. The optimal reduction in blood glucose level was observed during 2 h to 10 h. This might be due to the combined effect of gliclazide and *Emblica officinalis* on the release of insulin from beta cells of islet of Langerhans of pancreas in addition to their combined extrapancreatic effect [11]. *Emblica officinalis* stimulates the

secretion and action of insulin and inhibits starch digestion and protein glycation [12].

To check the validity of the observations seen earlier in normal rats, the studies were repeated on diabetic rats. The observations were considered to be significant if they exist in diabetic condition also. *Emblica officinalis* (300 mg/kg bd.wt) produced a significant anti-hyperglycaemic activity with peak activity at 3h. It was found to enhance the antihyperglycaemic activity of gliclazide during 2 to 10 h when administered in combination. The anti-hyperglyceamic activity of *Emblica officinalis* might be due to the presence of active constituents which were reported to produce antihyperglycaemic activity which was discussed above.

The hypoglycaemic effect was qualitatively similarly but quantitatively 2-8 % more in diabetic condition compared to matching control in rats.

CONCLUSIONS

The drug interaction studies of *Emblica officinalis* with gliclazide was conducted in normal and diabetic rats based on pharmacodynamic (blood glucose) response for 12 h. The normal rat model served to quickly identify the interaction and diabetic rat model served to validate the same response in actual use condition of drugs. In combination the extract of Emblica *officinalis* enhanced marginally the hypoglycaemic effect of gliclazide in normal rats and alloxan induced diabetic rats. The difference was statistically significant. The extract improved the hypoglycaemic activity of gliclazide because of pharmacodynamic mechanism only indicating similarity in their mechanism. The study provides scientific support for the use of the selected herbal extract in combination with gliclazide for better control of blood glucose and can be taken as positive interaction.

ACKNOWLEDGMENT

The authors are very much thankful to Dr. S. Satyanarayana, Principal, Avanthi Institute of Pharmaceutical Sciences, Cherukupally, Vizianagaram district, Andhra pradesh and the management of Gokaraju Rangaraju College of Pharmacy for their constant encouragement and support throughout the project work.

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Cite this article as:
M Ganga Raju, S Satyanarayana and Eswar Kumar.Improved efficacy of gliclazide with the aqueous extract of *emblica officinalis* on pharmacodynamic activity in normal and alloxan induced diabetic rats. J Pharm Chem Biol Sci 2014; 2(4)